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The Role of DNA Repair and the Epigenetic Markers Left after Repair in Neurologic Functions, Including Memory and Learning

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Abstract

In eukaryotic cell nuclei, DNA is wrapped around and firmly associated with histone proteins, forming chromatin. When DNA is damaged, the chromatin structure needs to be loosened to allow repair enzymes to gain access to the damage. This requires modifying the histone proteins. These modifications, called epigenetic alterations, do not alter the base-pair sequence. Repair-associated epigenetic alterations are usually transient, removed when no longer needed for repair. However, some remain after repair. In the human brain, long-lasting novel epigenetic alterations appear to account for the persistence of addictions to such substances as alcohol, nicotine and cocaine. Certain neurodegenerative diseases are caused by inherited mutations in genes necessary for DNA repair. Deficient DNA repair in these diseases is associated with extensive epigenetic alterations that likely have a role in the disease phenotype. Persistent epigenetic alterations due to DNA repair processes, both histone modifications and methylations of DNA, can also have positive consequences. Stimulation of brain activity (e.g. learning and memory formation) is often accompanied by the generation of DNA damage in neuronal DNA, followed by repair associated with persistent epigenetic alterations. In particular, recent research has shown the need for non-homologous end joining and base excision repair in memory formation.

Keywords: DNA repair, epigenetic, histone acetylation, histone methylation, CpG island methylation, addiction, neurodegenerative disease, memory, learning, cognition

1. Introduction

Even in the earliest stages of evolution, damage to the genome was presumably a fundamental problem for life. Thus it is likely that organisms developed processes for repairing genome damages very early. Such repair processes are ordinarily restorative, designed to reestablish the original undamaged genome sequence. During the course of the evolution of lineages leading to mammals, DNA repair processes became more complex, and acquired additional capabilities. One such example is the employment of the DNA double-strand break repair process of non-homologous end joining in the generation of immunological diversity [1].

In chromatin, epigenetic alterations are an integral part of DNA repair processes [2]. Although most epigenetic alterations introduced during DNA repair are transient with restoration of the epigenetic pattern that existed prior to repair, some are long lasting. Epigenetic alterations can enhance or inhibit gene expression without changing the DNA base pair sequence. Examples of epigenetic alterations are hyper- or hypomethylation of cytosines in the DNA sequence, increased or decreased histone H3 and H4 acetylation by histone acetyltransferases or histone deacetylases, and increased or decreased histone methylation by histone methyltransferases or histone demethylases.

In humans, the oxygen demands of the brain are high, constituting about 20% of total body oxygen consumption, while the mass of the brain is only about 2% of body mass [3]. This results in elevated release of reactive oxygen species in the brain that, in turn, cause oxidative DNA damages. Because damages are prevalent, processes that repair DNA damages have a vital role in maintaining the health of brain neurons, and these DNA repair processes can cause epigenetic alterations.

When DNA repair processes are impaired or insufficient, the result can be improper (non-adaptive) epigenetic alterations. Such improper epigenetic alterations in neurons are likely an important underlying cause of certain addictions and neurodegenerative diseases. Several addictive agents cause increased DNA damage in neurons resulting in increased dependence on DNA repair. Addictions are associated with characteristic persistent patterns of epigenetic alterations in the brain. In several neurodegenerative diseases the neurological impairments are caused by inherited mutations in genes that encode proteins employed in DNA repair. These diseases are associated with particular patterns of epigenetic alterations in neurons. It is likely that the neurological impairments suffered by individuals during addiction or neurodegenerative disease are caused, at least in part, by epigenetic alterations resulting from insufficient or faulty DNA repair. That is, insufficient or faulty DNA repair may produce epigenetic alterations that have long-lasting negative consequences at the level of gene expression that manifest as neurological impairment.

Zovkic et al. [4] noted that learning and memory can be broadly defined as lasting alterations of a behavioral output produced in response to a transient environmental input. In order for a brief stimulus to cause a persistent change in behavior, neurons need to undergo some kind of molecular alterations that stabilize a memory into an enduring set of cellular marks. As reviewed by Bird in 2002 [5], in mammals, DNA methylation is adapted for specific cellular memory in development, even over successive cell divisions. This observation of cellular memory indicated that epigenetic mechanisms could provide a molecular basis for neuronal memory formation and maintenance in non-replicating neurons [4]. In addition to DNA methylation/demethylation, it is now known that other mechanisms such as chromatin histone acetylation and histone methylation can also cause persistent epigenetic changes [6].

In the sections below, we review evidence for the following ideas. Neuronal activity causes DNA damages, and repair processes are required to deal with these damages. Such repair processes involve epigenetic alterations, some of which are long lasting. Individuals, addicted to abuse of certain substances that cause DNA damage, have long-lasting epigenetic alterations in brain neurons that appear to be related to the dependency. Also, inherited inability to adequately repair DNA damages can cause epigenetic alterations in neurons associated with neurodegenerative disease. However, long-lasting epigenetic alterations can also be adaptively beneficial. Cognitive functions such as memory and learning in response to external stimuli appear to depend, at least in part, on persistent epigenetic alterations arising during DNA repair processes.

2. Epigenetic alterations required for DNA repair

DNA is condensed in the nucleus of the cell in a highly organized and compact manner, referred to as chromatin (reviewed by Walker and Nestler [7] and Ding et al. [8]). In chromatin, the DNA is packaged with histone proteins to form nucleosomes. DNA repair proteins are recruited and interact with DNA in response to DNA damage. However, the architecture of nucleosomes and the organization of chromatin can present barriers to DNA damage recognition and repair. Epigenetic modifiers play an important role in regulating nucleosome and chromatin structure to facilitate DNA repair. Epigenetic alterations relax certain regions of chromatin to allow access to DNA repair enzymes and also condense certain regions to repress transcription in order to facilitate repair. When repair is complete, epigenetic modifications are largely returned to the state before damage occurred. These roles of epigenetic modifiers in DNA repair have been described as the “access-repair-restore” model [9].

2.1 Histone acetylation

The basic unit of chromatin, the nucleosome, is composed of 147 DNA base pairs wrapped around a histone octamer consisting of two copies of each of the following proteins: H2A, H2B, H3, and H4. The histones also have histone tail extensions, constituting up to 30% by mass of the histones (**Figure 1**). Each histone protein can undergo post translational modifications in which molecules, such as an acetyl group or one (or up to three) methyl group(s), are covalently added to (or removed from)

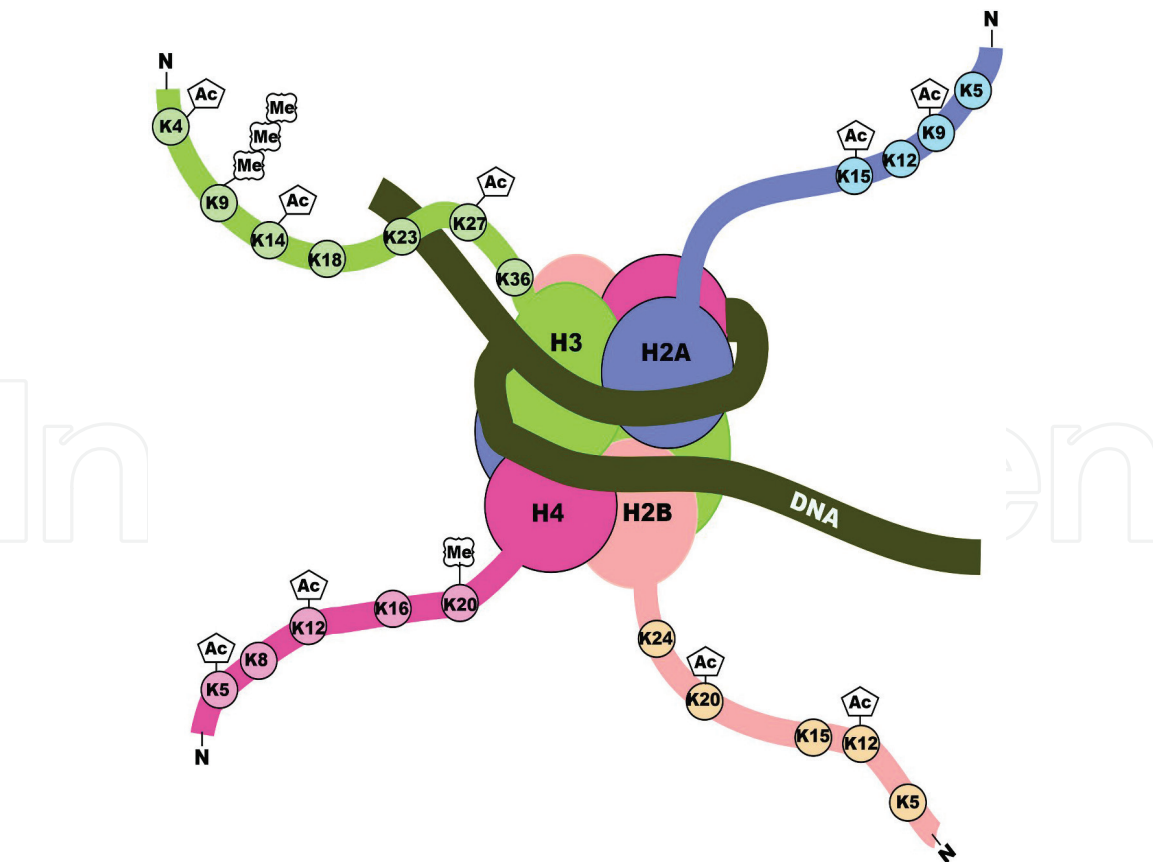


Figure 1.
A nucleosome showing 4 pairs of histones (H2A, H2B, H3, and H4), each pair with the same color. The amino-terminal (N-terminal) tails of one of each pair of histones is shown, labeled with the positions of lysine amino acids (labeled “K” in the single letter code for lysine) that are subject to acetylation or methylation. The number at each lysine indicates its position counting from the amino end of the protein chain. Acetylations (indicated by Ac) and methylations (indicated by Me) are shown in some positions susceptible to these alterations. The DNA, wound around the histone core, is indicated by the dark line.

lysine residues of their amino terminal (N-terminal) tail. The single letter K designates lysine. If an acetyl group is added to a lysine located as the 4th amino acid from the N-terminal tail end of histone 3, this is designated H3K4Ac. These modifications not only alter the structure of the nucleosome but also change the interaction of DNA with the associated histones, thus allowing entry of DNA repair enzymes into chromatin and permitting histones to be moved, if needed, to allow for repair [2]. The epigenetic modifications, if they remain after DNA repair, also can increase or decrease the likelihood of transcription of a given gene near the site of the repaired DNA damage [10].

Acetylation of histone lysines promotes chromatin relaxation to facilitate DNA repair [2]. It is also generally associated with a permissive transcriptional state. By negating the positive charge associated with the lysine residues on histone tails, acetylation promotes an “open” chromatin state.

2.2 Histone methylation

Histone lysine methylation is associated with either activation or repression of gene expression depending on which residues are methylated and whether one, two or three methyl groups are added at that position [11]. For instance enrichment of H3K4Me1 or H3K4Me3 at specific gene regions is correlated positively with increased transcription levels, whereas enrichment of H3K9Me2, H3K9Me3 or H3K27Me3 is negatively correlated with transcription. In response to DNA double-strand breaks, histone methyl transferases are recruited to sites of damage where they catalyze trimethylation of H3K9 and H3K27, thereby repressing transcription in order to facilitate DNA repair [12].

2.3 DNA methylation

An important epigenetic regulator in addition to histone modification is DNA methylation. DNA methylation often occurs with the addition of a methyl group to the DNA sequence cytosine-phosphate-guanine (CpG) at the C5 position (5mC). DNA methylation at gene promoters is generally associated with repression, while methylation within genes has been associated with active transcription [7]. DNA methyl transferase 1 (DNMT1) binds to sites of oxidative damage formed in GC-rich regions of the genome and promotes formation and recruitment of a large epigenetic silencing complex. Localization of these epigenetic modifiers to sites of oxidative damage in promoter CpG islands results in increased DNA methylation.

2.4 Noncoding RNA

Non-coding RNAs provide an additional type of epigenetic regulation. As one example, microRNAs are short sequences of RNA (about 22 bases) that exert a repressive role on gene expression by binding a target sequence on specific mRNAs and blocking translation or inducing degradation. The typical microRNA has about 400 specific target mRNAs. In one report, specific microRNAs collaborated with histone deacetylases and cooperatively regulated several relevant target genes [13].

2.5 Epigenetic alterations allow DNA repair

Figure 2 illustrates some of the actions of epigenetic alterations. Histone acetyltransferases add acetyl groups to histone tails to open chromatin structure to make DNA damages accessible to repair enzymes. If the acetyl groups remain after DNA repair, this allows genes in the area of DNA repair to be switched on. Histone deacetylases remove acetyl groups from histone tails to complete DNA repair and return chromatin to its condensed state existing before DNA repair. Improper actions of histone deacetylases can inappropriately switch genes off.

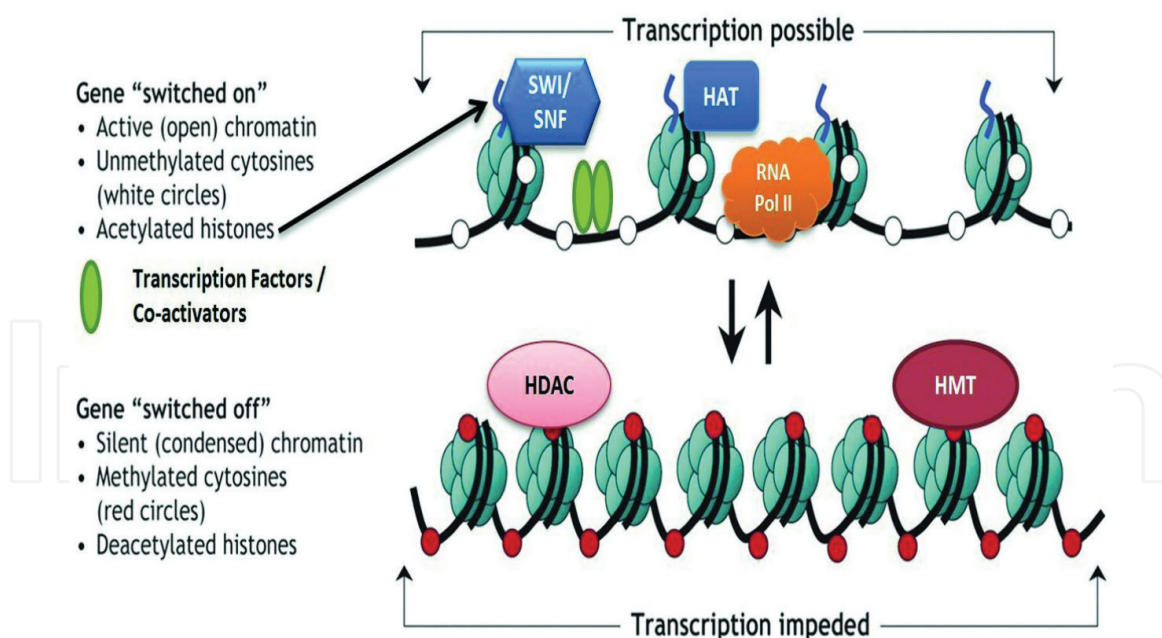


Figure 2.

A gene located in chromatin, with its DNA wrapped around histones. Open chromatin (top image) has been relaxed by acetylation of histone tails. Condensed chromatin (lower image) has been tightened by removal of acetyl groups and addition of methylation of histone tails. Symbols include histone acetyltransferase (HAT), histone deacetylase (HDAC), and histone methyltransferase (HMT). SWI/SNF (not an epigenetic alteration) is a nucleosome-remodeling complex that stimulates gene expression if the epigenetic factors (methylation on cytosines in DNA; acetylations and methylations of lysines in histone tails) allow transcription [14].

In this figure, histone methyltransferase (HMT) is shown as switching a gene off. However, some histone methylations serve to activate genes [11].

DNA gene promoters without methylated cytosines are indicated (in **Figure 2**) as allowing transcription (upper image), and DNA gene promoters with methylated cytosines (in CpG sites) are indicated as impeding transcription. The green vertical ovals represent transcription factors. They are not epigenetic factors, but also regulate gene transcription.

3. Epigenetic remnants (scars) after DNA repair

Dabin et al. [15], in an extensive review, noted that after repair of various types of DNA damages there are a number of types of epigenetic alterations that could potentially remain as scars. These epigenetic alterations include (1) changes in DNA methylation, (2) incorporation into nucleosomes of new histones with a pattern of acetylations or methylations that differ from that in the histones originally present before DNA damage, (3) incorporation into nucleosomes of histone variants such as histone H2AZ, (4) altered acetylation or methylation of histone tails, and even (5) altered histone density at repair sites. Several illustrative reports showing such scars after DNA repair are described below.

3.1 Homologous recombinational repair (HRR) of double-strand breaks leaves epigenetic alterations

Homologous recombinational repair (HRR) modified the DNA methylation pattern of a repaired DNA double-strand break in a green fluorescent protein (GFP) gene inserted into the HeLa cell genome [16]. In different subclones isolated after HRR repair events, the repair created either more highly methylated or less highly methylated cytosines in the GFP gene DNA. HRR also altered local histone H3 methylation,

forming H3K9Me2 or H3K9Me3 at repair locations. However, H3K9Me2/3 was selectively retained after HRR only in recombined genes with increased DNA methylation.

During a 2-week period after repair, some transcription-associated demethylation of the repaired DNA was promoted by base excision repair enzymes [16]. Subsequently, the repaired genes displayed stable but diverse methylation profiles. These profiles governed the levels of expression in each clone. These epigenetic alterations (scars) were stable over time and were recovered with the same frequency after 3 years of continuous culture.

3.2 Double-strand break repair by non-homologous end joining leaves epigenetic alterations

DNA repair by non-homologous end joining induces alterations in DNA cytosine methylation and these alterations are a source of permanent epigenetic changes [17]. In a HeLa cell line containing a green fluorescent protein (GFP) based reporter gene, a double-strand break in the gene followed by non-homologous end joining repair created two populations of cells, those with increased DNA methylation in the GFP gene (identified by a dim green color) and those with decreased DNA methylation in the GFP gene (identified by a bright green color). The degree of methylation for each population changed somewhat over the subsequent 4 days, but then remained stable for 24 days. Even though the HeLa cells were undergoing replication, the epigenetic changes produced stable high expressing or low expressing clones.

3.3 Base excision repair is associated with epigenetic alterations

The major forms of oxidative DNA damage are non-bulky lesions such as 8-oxo-2'-deoxyguanosine and thymine glycol that are repaired predominantly by base excision repair. After oxidative DNA damage was increased in HCT116 cells in culture, histone alterations were found in genes with CpG island-containing gene promoters and these histone alterations caused decreased transcription [18]. The histone alterations introduced by increased oxidative damage included reduction in H3K4Me3 and H4K16Ac and an increase in H3K27Me3. DNA methylation was also increased, but primarily in promoters of genes which normally have low basal expression [18].

3.4 Histone replacements during DNA repair

For many types of DNA damage, histones must be removed and replaced during the repair of the damaged DNA [19]. Disruption of nucleosomes in human cells after introduction of double-strand breaks or UV damage occurs with a drop in histone H2B levels and a selective loss of histones H2A and H2B, but not of H3 or H4 at the site of the damage [19]. After DNA repair, new histones (in addition to some pre-existing histones) are deposited at the site of repair. The new histones lack the histone post-translational modifications that existed before the repair. The presence of the differently modified new histones can specifically mark the domain as a site of repair, and remain as a scar [19]. The failure to recycle all of the pre-existing histone marks results in alterations in gene expression [15].

4. Addiction

One of the principal features of addiction is its persistence. The persistent behavioral changes appear to be due to long-lasting changes, resulting from epigenetic alterations affecting gene expression, within particular regions of the brain [20].

4.1 Alcohol

Alcohol can be addictive. About 7% of the US population are alcoholics, with alcohol use disorder [21]. Many negative physiologic consequences of alcoholism are reversible during abstinence. Long-term chronic alcoholics suffer a variety of cognitive deficiencies [22]. Multiyear abstinence resolves many neurocognitive deficits. One exception is lingering deficits in spatial processing [23]. In addition, some frequent long-term consequences are not reversible during abstinence. Alcohol craving (compulsive need to consume alcohol) is usually present long-term among alcoholics [24]. Among 461 individuals who sought help for alcohol problems, follow-up was provided for up to 16 years [25]. By 16 years, 54% of those who tried to remain abstinent without professional help had relapsed, and 39% of those who tried to remain abstinent with help (such as Alcoholics Anonymous) had relapsed.

Long-term, stable consequences of chronic alcohol abuse are thought to be due to stable changes of gene expression resulting from epigenetic alterations within particular regions of the brain [26–28]. For example, in rats exposed to alcohol for up to 5 days, there was an increase in histone 3 lysine 9 acetylation in the pronociceptin promoter in the brain amygdala complex. This acetylation is an activating mark for pronociceptin. The nociceptin/nociceptin opioid receptor system is involved in the reinforcing or conditioning effects of alcohol [29].

4.2 Cigarette smoking

Cigarette smokers (about 21% of the US population in 2013) [30] are usually addicted to nicotine [31]. This is a strong addiction. The proportion of smokers who reported having seriously tried to quit and who managed to quit for 6 months or more was less than 10% [32].

After 7 days of nicotine treatment of mice, the post-translational modifications consisting of acetylation of both histone H3 and histone H4 was increased at the *FosB* promoter in the nucleus accumbens of the brain, causing a 61% increase in *FosB* expression [33]. This also increases expression of the splice variant *Delta FosB*. In the nucleus accumbens of the brain, *Delta FosB* functions as a “sustained molecular switch” and “master control protein” in the development of an addiction [34, 35]. Similarly, after 15 days of nicotine treatment of rats, the post-translational modification consisting of threefold increased acetylation of histone H4 occurs at the promoter of the dopamine D1 receptor gene in the prefrontal cortex of the rats. This caused increased dopamine release in the prefrontal cortex reward-related brain region, and such increased dopamine release is recognized as an important factor for addiction [36].

4.3 Cocaine

Cocaine addiction occurs in about 0.5% of the US population. In humans treated for cocaine addiction, the relapse rate after 5 years was 25% [37]. Repeated cocaine administration in mice induces post-translational modifications including hyperacetylation of histone 3 (H3) or histone 4 (H4) at 1696 genes in one brain reward region, the nucleus accumbens, and deacetylation at 206 genes [7, 38]. At least 45 genes, shown in previous studies to be upregulated in the brain nucleus accumbens of mice after chronic cocaine exposure, were found to be associated with post-translational hyperacetylation of histone H3 or histone H4. Many of these individual genes are directly related to aspects of addiction associated with cocaine exposure [38].

4.4 Addictive substances can cause DNA damage

In rodent models, many addictive substances cause DNA damage in the brain. For example, alcohol, through its metabolic product acetaldehyde, induces double-strand breaks in DNA in the mouse brain [39].

Nicotine from cigarette smoke also very likely causes DNA damage in the brain. Nicotine reaches the brain 10–20 seconds after a puff of smoke. The level of nicotine in the brain is 75–80% as high as in the blood or the liver [40]. E-cigarette smoke is composed primarily of nicotine vapors. Nicotine from E-cigarettes, applied to mice (with the dose and duration equivalent in human terms to light E-cigarette smoking for 10 years), caused DNA damages including mutagenic O⁶-methyl-deoxyguanosines and γ -hydroxy-1,N₂-propano-deoxyguanosines in the lung, bladder, and heart [41]. These same damages are likely to occur as well in neurons upon exposure to nicotine.

Cocaine [42] and methamphetamine [43, 44] each also cause DNA damage in the brain.

After repair at the sites of DNA damages caused by drugs of addiction, the epigenome may not return entirely to their pre-damage states. Some of the methylations of DNA and/or the acetylations or methylations of histones at the sites of DNA repair may remain and thus become epigenetic scars on chromatin [15]. Such epigenetic scars likely contribute to the persistent epigenetic alterations found in addiction.

5. Neurodegenerative diseases with deficient DNA repair

DNA repair processes in mammalian cells normally involve extensive chromatin remodeling. This remodeling involves epigenetic modifications of chromatin that are usually transient, but may persist. When a protein necessary for proper DNA repair is mutationally defective, epigenetic alterations that deviate from a normal functional pattern can be introduced. In a number of neurodegenerative diseases, such epigenetic alterations appear to significantly underlie the disease phenotype.

We describe below four neurodegenerative diseases, ataxia telangiectasia, Huntington's disease, Aicardi-Goutières syndrome and Cockayne syndrome that have inherited genetic deficiencies due to mutations in genes necessary for DNA repair. We briefly summarize for each disease, the notable neurodegenerative features of the disease, the DNA repair processes that are defective, and the accompanying epigenetic alterations that likely have a role in the etiology of the disease. On the basis of the evidence reviewed, it appears that the proper functioning of the nervous system depends on DNA repair processes that not only restore damaged DNA sequence information, but also promote normal gene expression through the maintenance of an appropriate pattern of epigenetic markers.

5.1 Ataxia telangiectasia (AT)

AT is a multisystem disease characterized by neurodegeneration in the central nervous system. Certain regions of the brain including the cerebellum, are adversely affected in AT resulting in difficulty with movement and coordination. There is also an association with microcephaly. AT is inherited as an autosomal recessive trait, and is caused by mutation of the gene *AT mutated* (*ATM*) that encodes a serine/threonine protein kinase. The wild-type ATM protein has a key role in the DNA damage response. ATM is part of a molecular complex that signals the presence of oxidative DNA damage, including double-strand breaks, and facilitates subsequent repair [45].

ATM protein is employed in chromatin remodeling and in epigenetic alterations that are required for repairing DNA double-strand breaks [45]. ATM mutation causes defects in epigenetic regulation that likely contribute to the rapid postnatal degeneration of the cerebellum that underlies the progressive ataxia observed in AT [45]. AT is associated with histone acetylation alterations, including significant decreases in histone H3 and H4 acetylation [46]. ATM regulates neuron specific epigenetic alterations involving histone deacetylase-4 [45]. In ATM mutant neurons, misallocation of histone deacetylase-4 represses transcription of genes important in neuronal function and synaptic maintenance [45].

5.2 Huntington's disease (HD)

HD typically occurs in midlife. The symptoms include progressive movement disorder, cognitive dysfunction and psychiatric impairment. HD is inherited in an autosomal dominant manner. HD results from an unstable expansion of CAG repeat sequences in exon 1 of the *huntingtin* gene (*HTT*). Several lines of evidence link the *HTT* protein to repair of DNA damage [47]. *HTT* is a scaffolding protein that directly participates in oxidative DNA damage repair [48]. The ATM protein recruits *HTT* to sites of DNA damage. *HTT* co-localizes with, and acts as a scaffold for, proteins of the DNA damage response pathway in response to oxidative stress. The fibroblasts of HD patients with expanded CAG repeats have deficient oxidative damage repair [48].

Impaired DNA repair in HD also appears to cause deleterious epigenetic alterations that are linked to transcriptional dysregulation. Individuals with HD experience accelerated epigenetic aging of the brain, particularly in the frontal lobe, cingulate gyrus and the parietal lobe. This process is associated with substantial changes in brain DNA methylation levels [49]. Also post-translational modifications of histone proteins are significantly altered in HD patients as well as in HD cellular and animal models [50].

5.3 Aicardi-Goutières syndrome (AGS)

AGS is characterized by early onset, often in early infancy. Features of AGS include neurological dysfunction, psychomotor retardation, seizures, and microcephaly [51]. AGS is an inherited disease and most cases are inherited in an autosomal recessive pattern. AGS arises from mutations in genes encoding proteins TREX 1 (AGS1), RNase H2 (AGS2, 3 and 4) and SAMHD1 (AGS5) [51]. The incorporation of ribonucleotide triphosphates (rNTPs) into DNA is perhaps the most common type of endogenous DNA damage encountered in proliferating cells [52]. Removal of rNTPs incorporated into DNA is referred to as rNTP excision repair. Key players in rNTP excision repair are TREX1 and RNase H2 [52]. RNase H2 is the predominant nuclear enzyme to hydrolyze the RNA strand of RNA/DNA hybrids [53].

TREX1, *RNASEH2* and *SAMHD1* mutations in AGS cells cause common molecular abnormalities including increased levels of RNA:DNA hybrid species and genome-wide DNA hypomethylation, a substantial epigenetic perturbation [51]. AGS2 and AGS4 mutant cells display about a 20% reduction in genomic methylation levels overall, and this reduction is spread along the length of entire chromosomes impacting nearly all compartments including genic, intergenic unique and repeat regions [51].

5.4 Cockayne syndrome (CS)

Due to impaired neurological development, individuals with CS are characteristically mentally retarded and have microcephaly. CS is caused by mutations in the *CSA* and *CSB* genes. CS is inherited as an autosomal recessive trait. Transcription of DNA can be inhibited by DNA damage, and restoration of transcription requires removal

of blocking damages by a sub-pathway of nucleotide excision repair that specifically removes transcription-blocking DNA damages. This sub-pathway is referred to as transcription-coupled DNA repair (TCR). In mammals, TCR depends on the CSA and CSB proteins. More than 70% of CS syndrome patients have a mutation in the *CSB* gene. CSA and CSB proteins regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase at sites of DNA damage [54, 55].

Among the proteins recruited by CSA and CSB are nucleotide excision repair proteins and histone acetyltransferase, an enzyme that catalyzes chromatin remodeling and epigenetic alteration [54]. CSB can slide histones along DNA and histone chaperone proteins that accept and donate histones can greatly facilitate this process [55]. Nucleosome remodeling by CSB is important for TCR, and inability to efficiently mobilize nucleosomes appears to contribute to the underlying mechanism of CS [55]. The chromatin remodeling activity of CSB appears to create an epigenetic landscape that permits more efficient DNA repair or facilitates transcription resumption after repair is completed [56].

5.5 Perspective on the role of DNA repair in neurodegeneration

The neurodegenerative diseases AT, HD, AGS and CS are due to mutation in genes that encode proteins employed in DNA repair. Inadequate DNA repair can lead directly to cell death and neuron depletion that may be reflected in microcephaly, as is seen in AT, AGS and CS. The defects in DNA repair also cause disruptions in the pattern of epigenetic alteration required for normal neuronal function. These epigenetic alterations likely underlie characteristic features of the disease phenotype. Thus it appears that important functions of the nervous system, including those involved in various aspects of cognition and motor function, depend on the role of intact DNA repair processes in maintaining normal patterns of epigenetic markers.

5.6 Other neurodegenerative diseases deficient in DNA repair

In addition to the four neurodegenerative diseases discussed above, there is also evidence for defective DNA repair in the neurodegenerative diseases amyotrophic lateral sclerosis [57], fragile X syndrome [58], Friedrich's ataxia [59], spinocerebellar ataxia type 1 [60], trichothiodystrophy [61], and xeroderma pigmentosum [62].

Amyotrophic lateral sclerosis is causally linked to mutations in the gene *FUS* [57]. ALS patients with *FUS* mutations have increased neuronal DNA damage. FUS protein functions in the DNA damage response including recruitment to double-strand breaks and homologous recombinational DNA repair. FUS protein also directly interacts with histone deacetylase 1 in response to DNA damage, and this interaction is necessary for efficient DNA repair [57].

Fragile X syndrome is a common form of inherited mental retardation. The fragile X mental retardation protein FMRP is a chromatin-binding protein that functions in the DNA damage response, likely in DNA repair [58]. Fragile X syndrome is caused by loss of expression of the *FMR1* gene, most often due to an expansion of a CGG repeat in the first exon of *FMR1*. The repeat expansion results in abnormal methylation of the promoter region which leads to transcriptional silencing of the *FMR1* gene [63].

Friedreich ataxia, a progressive neurodegenerative disease, is caused by deficient frataxin protein resulting from downregulation of the *FXN* gene. Frataxin is employed in the repair of DNA double-strand breaks [59]. Most individuals with Friedrich ataxia have a homozygous mutation consisting of a GAA trinucleotide repeat expansion within the first intron of the *FXN* gene. This expansion itself may lead to downregulation of the *FXN* gene. In addition, there is a repressive

heterochromatin effect around the *FXN* gene caused by the expanded GAA repeats, consisting of high levels histone methylation of H3K9 and H3K27 [64].

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease characterized by progressive motor incoordination. SCA1 results from mutation in the *ATXN1* gene that leads to a pathogenic glutamine-repeat expansion in the protein ataxin-1 (ATXN1). The multifunctional protein TERA/VCP/p97 acts in DNA damage repair. Glutamine-repeat expansion mutant proteins such as mutant ATXN1 impair accumulation and function of TERA/VCP/p67 leading to an increase in unrepaired DNA double-strand breaks [60]. Also mutant *ATXN1* represses gene transcription by decreasing histone acetylation [65].

Trichothiodystrophy (TTD) is an autosomal recessive disorder with a range of clinical neurodevelopmental features and often photosensitivity. All photosensitive TTD individuals have a mutation in the *XPB*, *XPD* or *TTDA* genes that encode subunits of the dual functional repair/transcription factor IIH (TFIIH) [61]. These individuals deficient for TFIIH are defective in nucleotide excision repair, a process that repairs transcription-blocking DNA damages, including UV induced DNA damages, thus explaining their photosensitivity. Induction of DNA damage in cells with *XPB* or *XPD* mutations that cause TTD results in reduced transient DNA strand breaks that are intermediates during DNA repair [66]. Also methylation of histone H3 (H3K9Me3) was reduced in an evaluated model promoter region [66].

Xeroderma pigmentosum (XP) is an autosomal recessive genetic disorder. XP has characteristic neurological manifestations, but the most prominent feature of the condition is sensitivity to sunlight resulting in a high predisposition to UV-induced skin cancer. Seven different complementation groups (genes) *XPA*, *XPB*, *XPC*, *XPD*, *XPE*, *XPF* and *XPG* encode proteins employed in nucleotide excision repair, a process that repairs bulky DNA damages including damages caused by UV-light [62]. *XPF* and *XPG* proteins are endonucleases that also trigger chromatin looping and DNA demethylation that promote accurate expression of activated genes [67].

6. Mental activity is associated with DNA damage and repair in the brain

An easy type of DNA damage to measure is the double-strand break. When a double-strand break occurs there is a rapid effect on particular histones near the break. A variant histone, H2AX, is sometimes present in histone cores, and it constitutes about 2–25% of the H2A histones in mammalian chromatin [68]. After a double-strand break, H2AX histones near the break are phosphorylated by the kinases ATM, ATR and DNA-PK [69], allowing formation of H2AX phosphorylated on serine 139 near the break. This histone is then designated γ H2AX. γ H2AX can be detected as soon as 20 seconds after irradiation of cells (with DNA double-strand break formation), and half maximum accumulation of γ H2AX occurs in 1 minute [68]. Chromatin with phosphorylated γ H2AX extends to about a million base pairs on each side of a DNA double-strand break [68]. It is easy to detect γ H2AX by immunohistochemistry, and these large segments of chromatin with γ H2AX are called γ H2AX foci.

Learning and new memories occur when mice explore a new, strange environment. This is a low level stimulation. Exploration of a novel environment increased the number of neurons with double-strand breaks in neuronal DNA as measured by γ H2AX foci [70]. This occurs in different brain regions but particularly in the dentate gyrus, which is involved in spatial learning and memory. Within 24 hours of break formation, DNA repair occurs with removal of the breaks [70]. When double-strand breaks in this situation were also measured by the comet assay (another simple assay), roughly 30–40% of dentate gyrus nuclei had comet tails indicating double-strand breaks in the nuclear DNA [70].

6.1 Visual stimulation

Another neuronal activity also caused double-strand breaks. Exposure of anesthetized mice to visual stimuli activated the primary visual cortex (V1) of the brain. One eye was exposed to visual stimuli for 15 minutes, while the other was shielded from light. One hour after the visual stimulation began, the number of cells with γ H2AX foci in the stimulated contralateral V1 was roughly twice as high as that in the unstimulated ipsilateral V1 [70].

6.2 Optogenetic stimulation

Optogenetic stimulation of a mouse striatum brain region also caused DNA double-strand breaks [70]. Transgenic mice expressing Cre-recombinase in medium spiny neurons of the dorsomedial striatum were used. The Cre-recombinase gene inserted into DNA of the striatum neurons in these mice provides a topoisomerase I like mechanism to carry out site-specific recombination events. Using this system, a viral vector was infused into the striatum, carrying a genetic segment coding for a light sensitive Chr2 protein. The Chr2 gene frequently recombined into the mouse dorsomedial striatum DNA. A glass fiber was then implanted close to the viral injection site. Two weeks later, awake mice were stimulated by light through the glass fiber. This caused neuronal activity in the dorsomedial striatum, resulting in behavioral ipsiversive rotations in mice (mice turning in a circle). The mice were then terminated and the mouse brains examined. The illuminated striata contained many more cells with γ H2AX foci than the non-illuminated contralateral striata [70].

6.3 Non-homologous end joining (NHEJ) repair required for long-term memory retention

One form of long-term memory, through associative learning, is contextual fear conditioning [71]. This fear conditioning occurs, for instance, when a rodent is placed in a novel environment (a new context) and is then subjected to an electric shock (e.g. a footshock). This produces robust fear learning, shown by a strong fear response, when the rodent is placed in that context again. Contextual fear conditioning occurs very rapidly (can occur with a single event) and has a lasting effect.

Madabhushi et al. [72] subjected wild-type C57BL/6 mice to a training paradigm for contextual fear conditioning, following which they prepared hippocampal lysates and measured γ H2AX levels (as a measure of double-strand breaks in DNA). Elevated γ H2AX levels were detectable in hippocampal lysates within 15 minutes after exposure to the fear-conditioning paradigm.

NHEJ, which repairs double-strand breaks in DNA, appears to be needed specifically for consolidation of memory into long-term memory. Contextual fear conditioning in mice increased NHEJ repair activity in the hippocampus brain region measured at 10 and 60 minutes after training [73]. The hippocampus is important in forming memories [74].

When NHEJ repair was active, memories were demonstrated in fear-conditioned mice at 6 and 24 hours after training. Ara-C (cytosine arabinoside) interferes with DNA synthesis. Injecting animals systemically with ara-C 1 hour before exposing them to the conditioning inhibited NHEJ repair [68]. If NHEJ repair was blocked before fear conditioning, memories of fear conditioning were substantially diminished at the 6- and 24-hour time periods tested. Thus it appears that NHEJ repair is required for memory formation. Other cognitive elements were not blocked by ara-C. Mice given ara-C and then subjected to contextual fear conditioning maintained their short-term memory (tested at 30 seconds after training) and exploratory

behavior in an open field 24 hours after training. Treatment with ara-C also did not cause general malaise, motor in-coordination, sedation, or anxiety.

7. Long-term memory depends on epigenetic alterations

7.1 Contextual fear memory conditioning causes changes in DNA methylation in brain neurons

Halder et al. [75], in a mouse study, evaluated differently expressed genes and short differentially methylated regions in neurons of the anterior cingulate cortex, a brain region important for associative memory acquisition and maintenance of long-term memory. In the anterior cingulate cortex at 1 hour after contextual fear conditioning, there were 6250 differentially methylated genes with 46,395 differentially methylated short regions (700 base pair regions). (Frequently, multiple short differentially methylated regions occurred in a differentially methylated gene.) At 4 weeks after training 1223 differentially methylated genes and 5018 differentially methylated short regions persisted. In addition, at 4 weeks after training they found 1700 differentially expressed genes in the anterior cingulate cortex. These findings suggest that long-term memory (4 weeks) is associated with differential methylation of DNA and altered expression of genes.

Halder et al. [75] also evaluated differentially methylated regions and differently expressed genes in the hippocampal CA1 region, a region that is crucial for short-term memory formation during contextual fear conditioning. They found that, in contrast to the anterior cingulate cortex, in the hippocampus there were 1619 differentially methylated regions after 1 hour, but these changes did not persist, and almost none were present after 4 weeks.

Also studying the hippocampus, Duke et al. [76], working with rats, found that at 24 hours after contextual fear conditioning there were more than 5000 differentially methylated regions (500 base pair short regions), but less than 20 differentially methylated regions after context change alone. Hypermethylated differentially methylated regions overlapping differentially expressed genes were associated with decreased gene expression, consistent with the concept that cytosine methylation is often a mechanism for suppressing transcription. Also at 24 hours after contextual fear conditioning, there were more than 2000 differentially methylated regions that were associated with 1048 genes having down-regulated expression and 564 genes having up-regulated expression (usually known to be associated with hypomethylated regions). At 24 hours after training, 9.17% of the genes in the rat genome of hippocampus neurons were differentially methylated. Gene Ontology term analysis was performed, and differentially expressed gene enrichment analysis revealed that many of the genes involved in synaptic functions after fear conditioning were up-regulated.

7.2 The role of base excision repair in memory consolidation

In both the studies of Halder et al. [75] and Duke [76], above, there were on the order of a thousand demethylations of cytosines in neuron genomes during memory consolidation in the brain after contextual fear conditioning. The two likely processes of demethylating cytosine each depend on base excision repair, as shown in **Figure 3**. These processes were reviewed by Bayraktar and Kreutz [77]. There is considerable evidence for the left hand process illustrated in **Figure 3**. In this process there are two or more fast oxidations by one of the ten-eleven translocation methylcytosine dioxygenases (TET1, TET2, TET3), first altering 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), and then producing 5-formylcytosine (5fC) followed by

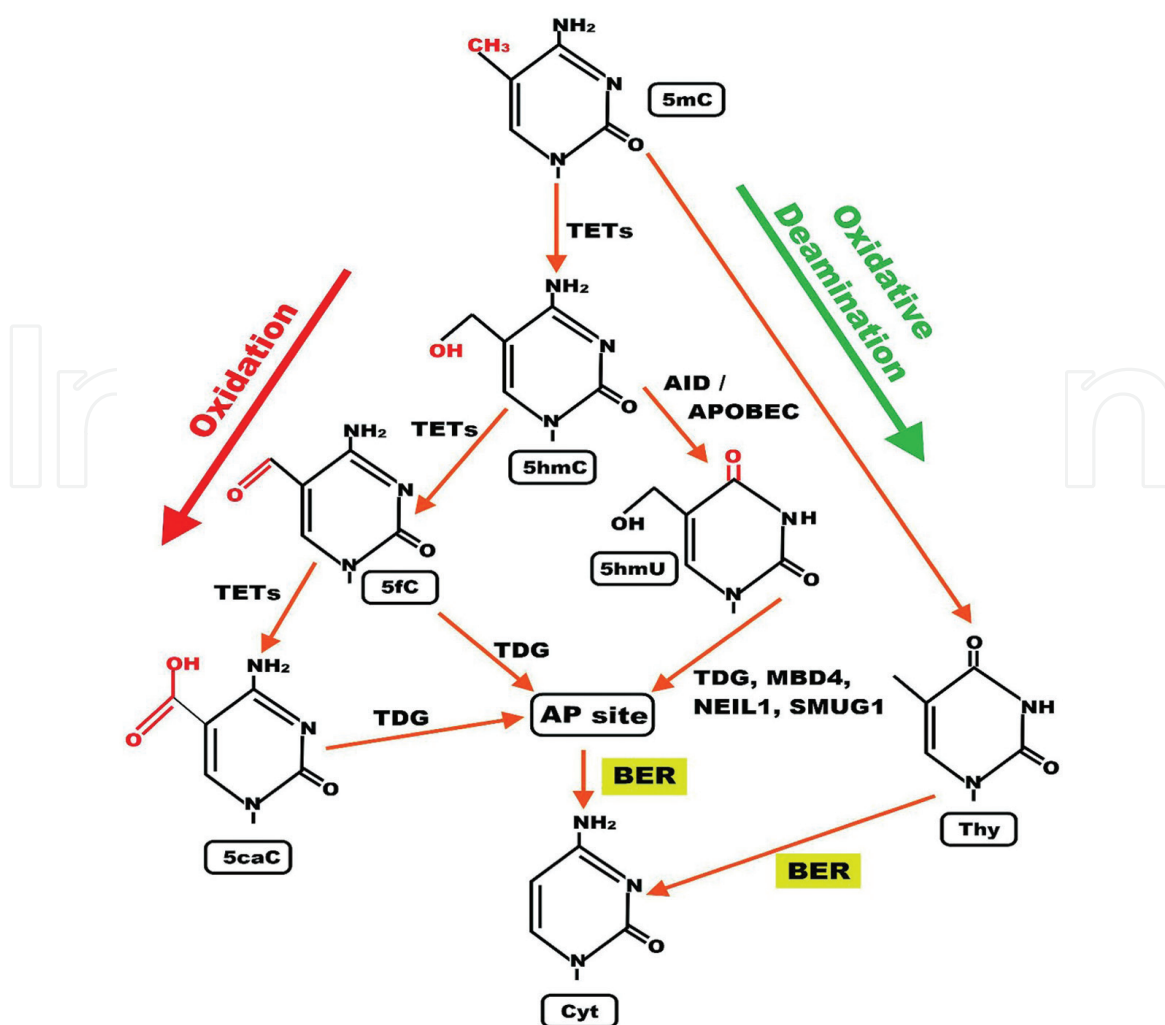


Figure 3.

Demethylation of 5-methylcytosine (5mC) to cytosine (Cyt) in DNA depends on base excision repair (BER) as the final step. In initial steps, the ten-eleven translocation methylcytosine dioxygenase family of enzymes (TET1, TET2, and TET3) each may catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and further steps form 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). 5fC and 5caC can be excised from DNA by thymine DNA glycosylase (TDG) to form an apyrimidinic site (AP site). AID/APOBEC is a family of cytidine deaminases that can oxidatively deaminate 5mC to 5-hydroxymethyl uracil (5hmU) or 5mC to thymine (Thy). 5hmU can be excised from DNA by TDG, methyl-CpG-binding domain protein 4 (MBD4), endonuclease VIII-like 1 (NEIL1) or single-strand selective monofunctional uracil DNA glycosylase (SMUG1).

5-carboxylcytosine (5caC). Both 5fC and 5caC can be excised by thymine DNA glycosylase (TDG), generating an apyrimidinic (AP) site, which is repaired by base excision repair to place cytosine (cyt) in the DNA opposite guanine. However there is some indication that a cytidine deaminase (AID/APOBEC) enzyme can carry out oxidative deamination of 5mC to 5-hydroxymethyluracil, which is then excised by one of the four enzymes shown, to form an AP site. Alternatively, a cytidine deaminase (AID/APOBEC) may carry out an oxidative deamination of 5mC by to thymine, and the mispair of thymine with guanine is then repaired by base excision repair to generate cytosine paired with guanine in DNA.

Zhang et al. [78] generated homozygous mutant mice deficient in TET1 catalytic activity. These mice were viable and fertile, with no discernible morphological or growth abnormality. The Tet1 deficient mice would be expected to have reduced ability to convert 5mC to cytosine by the TET/base excision repair-dependent pathway. When examined in neural progenitor cells, 478 genes showed elevated promoter DNA methylation levels compared to the wild-type control, while only 32 genes had lower DNA methylation. There was a link between the altered DNA methylation pattern and transcriptional activity. In the neural progenitor cells of TET1 mutant

mice 1267 genes were down-regulated with respect to transcription and 498 were up-regulated compared to wild-type. In particular, with TET1 mutant mice, 39 genes were found to be both hyper-methylated and down-regulated in neural progenitor cells isolated from the *dentate gyrus* (part of the brain hippocampus). Four-month-old wild-type and TET1 knockout mice were tested in the Morris water maze. The TET1 deficient mice, with reduced ability to use a pathway dependent on base excision repair, showed impairment in spatial learning and short-term memory.

8. Perspective on the role of DNA repair in cognitive functions

The evidence discussed above in Section 6 clearly indicated that neuronal activity causes DNA double-strand breaks, especially in early response genes after neuronal stimulation. NHEJ repair is required to repair these breaks, and NHEJ repair is required for long-term memory formation. As discussed in Section 7, long-term memory formation depends on large numbers of epigenetic alterations including methylations and demethylations of cytosine in DNA. Although it is known that repair of double-strand breaks by NHEJ repair can leave epigenetic alterations (scars) (including alterations in the pattern of cytosine methylation) after the repair occurs, it is not known whether the NHEJ repair “scars” are a major portion of these epigenetic alterations. About a thousand demethylations occur during long-term memory formation in rats and mice. Base excision repair is central to demethylation of 5mC to cytosine. A deficiency in the TET/base excision repair pathway causes diminished epigenetic demethylations of DNA as well as alterations in memory.

Overall, memory and learning depend on epigenetic alterations. Two forms of DNA repair, NHEJ repair and base excision repair, have essential roles in cognitive functions, and at least base excision repair has a direct role in regulating one major type of epigenetic alteration, the demethylation of 5mC to cytosine in DNA during memory formation.

9. Conclusion

In eukaryotic cell nuclei, DNA is associated with histone proteins in highly organized and compact structures to form chromatin. When the DNA is damaged, repair enzymes need to gain access to the damage, and this requires modification of the compact structure. These modifications, termed epigenetic alterations, include acetylation of histones, methylation of histones and methylation of CpG sequences in DNA. Such epigenetic alterations can allow access of repair enzymes to sites of DNA damage while not disturbing the DNA base-pair sequence.

DNA repair processes are characteristically initiated rapidly and completed in a short period of minutes to hours, but epigenetic alterations introduced by such repair may be retained after repair is completed. A type of epigenetic alteration that can last after repair of a double-strand break is the DNA methylation of CpG islands in gene promoters. Such epigenetic alterations can silence gene expression. Also, several types of oxidative DNA damage are removed by base excision repair. Base excision repair is accompanied by epigenetic alterations of histones that are associated with genes containing CpG islands in their promoters. These epigenetic alterations can cause decreased transcription of the genes.

The persistent behavioral changes that are a prominent feature of addictions appear to be the result of epigenetic alterations that affect gene expression in particular regions of the brain. Specific epigenetic alterations have been found to be associated with addiction to alcohol, nicotine and cocaine. The epigenetic alterations that occur in those particular regions of the brain are considered to be

involved with each of the addictions. Nicotine and cocaine, and alcohol through its metabolic product acetaldehyde, cause DNA damage in the brain. Such DNA damage is subject to DNA repair processes that likely cause at least a portion of the long lasting epigenetic alterations found in the brains of addicted individuals.

In humans and other mammals inherited mutations in genes necessary for DNA repair can cause neurodegenerative diseases. Examples of such diseases are ataxia telangiectasia, Huntington's disease, Aicardi-Goutières syndrome and Cockayne syndrome. The deficiencies in DNA repair in these diseases cause disruptions in the pattern of epigenetic alterations required for normal neuronal function. These epigenetic alterations likely underlie key features of the neurodegenerative disease phenotypes.

Learning and new memories occur when mice explore a new, strange environment. Exploration of a novel environment increases the number of neurons with double-strand breaks in neuronal DNA, particularly in the dentate gyrus, which is involved in spatial learning and memory. Another neuronal activity, visual stimulation, was found to cause DNA double-strand breaks. Direct stimulation of the striatum region of the brain also caused DNA double-strand breaks. Memory retention of context associated electric shock events in mice involved induction of double-strand breaks and their repair by the process of non-homologous end joining in the hippocampus, a region of the brain known to be important in forming memories. Inhibition of non-homologous end joining substantially diminished memory retention.

The anterior cingulate cortex is a brain region important for long-term memory formation. Long-term memory (4 weeks in mice) subsequent to a contextual conditioning experience was found to be associated with substantial retention of a differential DNA methylation and gene expression pattern in the anterior cingulate cortex. In addition, differential DNA methylation in the hippocampus appears to be associated with short-term memory formation. Together, long-term and short-term memory formations are associated with on the order of a thousand demethylations of cytosines in neuron genomes during memory consolidation. Demethylation of 5-methylcytosine to cytosine in DNA depends on base excision repair.

In general, the evidence indicates that, in mammals, DNA repair processes can cause epigenetic alterations in chromatin, some of which are long lasting. These epigenetic alterations can have negative consequences on neurological function such as in certain addictions and neurodegenerative diseases. In addition, epigenetic alterations resulting from DNA repair processes, such as non-homologous end joining and base excision repair, appear to have a positive role in facilitating adaptive cognitive capabilities that include memory and learning.


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References

- [1] Pryor JM, Conlin MP, Carvajal-Garcia J, Luedeman ME, Luthman AJ, Small GW, et al. Ribonucleotide incorporation enables repair of chromosome breaks by nonhomologous end joining. *Science*. 2018;**361**(6407):1126-1129. DOI: 10.1126/science.aat2477
- [2] Dhar S, Gursoy-Yuzugullu O, Parasuram R, Price BD. The tale of a tail: Histone H4 acetylation and the repair of DNA breaks. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2017;**372**(1731). pii: 20160284). DOI: 10.1098/rstb.2016.0284
- [3] Raichle ME, Gusnard DA. Appraising the brain's energy budget. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;**99**(16):10237-10239. DOI: 10.1073/pnas.172399499
- [4] Zovkic JB, Guzman-Karissom MC, Sweatt JD. Epigenetic regulation of memory formation and maintenance. *Learning & Memory*. 2013;**20**(2):61-74. DOI: 10.1101/lm.026575.112
- [5] Bird A. DNA methylation patterns and epigenetic memory. *Genes & Development*. 2002;**16**(1):6-21. DOI: 10.1101/gad.947102
- [6] Budhavarapu VN, Chavez M, Tyler JK. How is epigenetic information maintained through DNA replication? *Epigenetics & Chromatin*. 2013;**6**(1):32. DOI: 10.1186/1756-8935-6-32
- [7] Walker DM, Nestler EJ. Neuroepigenetics and addiction. *Handbook of Clinical Neurology*. 2018;**148**:747-765. DOI: 10.1016/B978-0-444-64076-5.00048-X
- [8] Ding N, Maiuri AR, O'Hagan HM. The emerging role of epigenetic modifiers in repair of DNA damage associated with chronic inflammatory diseases. *Mutation Research—Reviews in Mutation Research*. Online Sept 28, 2017. In Press. DOI: 10.1016/j.mrrev.2017.09.005
- [9] Polo SE, Almouzni G. Chromatin dynamics after DNA damage: The legacy of the access-repair-restore model. *DNA Repair (Amst)*. 2015;**36**:114-121. DOI: 10.1016/j.dnarep.2015.09.014
- [10] Eberharder A, Becker PB. Histone acetylation: A switch between repressive and permissive chromatin. Second in review series on chromatin dynamics. *EMBO Reports*. 2002;**3**(3):224-229. DOI: 10.1093/embo-reports/kvf053
- [11] Black JC, Van Rechem C, Whetstine JR. Histone lysine methylation dynamics: Establishment, regulation, and biological impact. *Molecular Cell*. 2012;**48**(4):491-507. DOI: 10.1016/j.molcel.2012.11.006
- [12] Wei S, Li C, Yin Z, Wen J, Meng H, Xue L, et al. Histone methylation in DNA repair and clinical practice: New findings during the past 5-years. *Journal of Cancer*. 2018;**9**(12):2072-2081. DOI: 10.7150/jca.23427
- [13] Swierczynski S, Klieser E, Illig R, Alinger-Scharinger B, Kiesslich T, Neureiter D. Histone deacetylation meets miRNA: Epigenetics and post-transcriptional regulation in cancer and chronic diseases. *Expert Opinion on Biological Therapy*. 2015;**15**(5):651-664. DOI: 10.1517/14712598.2015.1025047
- [14] Wikipedia article “Chromatin remodeling” figure is adapted from Luong, P. Basic Principles of Genetics, Connexions Web site (2009) under a Creative Commons Attribution License (CC-BY 3.0). Further modification of the figure is performed by the image uploader with reference from Davis PK, Brackmann RK. Chromatin

remodeling and cancer. *Cancer Biology & Therapy*. 2003;**2**:22. Image labeled for non-commercial reuse, licensed under the Creative Commons Attribution 3.0 License

[15] Dabin J, Fortuny A, Polo SE. Epigenome maintenance in response to DNA damage. *Molecular Cell*. 2016;**62**(5):712-727. DOI: 10.1016/j.molcel.2016.04.006

[16] Russo G, Landi R, Pezone A, Morano A, Zuchegna C, Romano A, et al. DNA damage and repair modify DNA methylation and chromatin domain of the targeted locus: Mechanism of allele methylation polymorphism. *Scientific Reports*. 2016;**6**:33222. DOI: 10.1038/srep33222

[17] Allen B, Pezone A, Porcellini A, Muller MT, Masternak MM. Non-homologous end joining induced alterations in DNA methylation: A source of permanent epigenetic change. *Oncotarget*. 2017;**8**(25):40359-40372. DOI: 10.18632/oncotarget.16122

[18] O'Hagan HM, Wang W, Sen S, Destefano Shields C, Lee SS, Zhang YW, et al. Oxidative damage targets complexes containing DNA methyltransferases, SIRT1, and polycomb members to promoter CpG Islands. *Cancer Cell*. 2011;**20**(5):606-619. DOI: 10.1016/j.ccr.2011.09.012

[19] Hauer MH, Gasser SM. Chromatin and nucleosome dynamics in DNA damage and repair. *Genes & Development*. 2017;**31**(22):2204-2221. DOI: 10.1101/gad.307702.117

[20] Nestler EJ, Barrot M, Self DW. DeltaFosB: A sustained molecular switch for addiction. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**(20):11042-11046. DOI: 10.1073/pnas.191352698

[21] Available from: <https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-facts-and-statistics>

[22] Oscar-Berman M, Valmas MM, Sawyer KS, Ruiz SM, Luhar RB, Gravitz ZR. Profiles of impaired, spared, and recovered neuropsychologic processes in alcoholism. *Handbook of Clinical Neurology*. 2014;**125**:183-210. DOI: 10.1016/B978-0-444-62619-6.00012-4

[23] Fein G, Torres J, Price LJ, Di Sclafani V. Cognitive performance in long-term abstinent alcoholic individuals. *Alcoholism, Clinical and Experimental Research*. 2006;**30**(9):1538-1544. DOI: 10.1111/j.1530-0277.2006.00185.x

[24] Bottlender M, Soyka M. Impact of craving on alcohol relapse during, and 12 months following, outpatient treatment. *Alcohol and Alcoholism*. 2004;**39**(4):357-361. DOI: 10.1093/alcalc/agh073

[25] Moos RH, Moos BS. Rates and predictors of relapse after natural and treated remission from alcohol use disorders. *Addiction*. 2006;**101**(2):212-222. DOI: 10.1111/j.1360-0443.2006.01310.x

[26] Krishnan HR, Sakharkar AJ, Teppen TL, Berkel TD, Pandey SC. The epigenetic landscape of alcoholism. *International Review of Neurobiology*. 2014;**115**:75-116. DOI: 10.1016/B978-0-12-801311-3.00003-2

[27] Jangra A, Sriram CS, Pandey S, Choubey P, Rajput P, Saroha B, et al. Epigenetic modifications, alcoholic brain and potential drug targets. *Annals of Neurosciences*. 2016;**23**(4):246-260. DOI: 10.1159/000449486

[28] Berkel TD, Pandey SC. Emerging role of epigenetic mechanisms in alcohol

addiction. Alcoholism, Clinical and Experimental Research. 2017;**41**(4): 666-680. DOI: 10.1111/acer.13338

[29] D'Addario C, Caputi FF, Ekström TJ, Di Benedetto M, Maccarrone M, Romualdi P, et al. Ethanol induces epigenetic modulation of prodynorphin and pronociceptin gene expression in the rat amygdala complex. Journal of Molecular Neuroscience. 2013;**49**(2):312-319. DOI: 10.1007/s12031-012-9829-y

[30] Available from: <https://www.samhsa.gov/data/sites/default/files/NSDUHresultsPDFWHTML2013/Web/NSDUHresults2013.pdf>

[31] Available from: <https://www.drugabuse.gov/publications/research-reports/tobacco-nicotine-e-cigarettes/nicotine-addictive>

[32] Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2376894/pdf/0980317.pdf>

[33] Levine A, Huang Y, Drisaldi B, Griffin EA Jr, Pollak DD, Xu S, et al. Molecular mechanism for a gateway drug: Epigenetic changes initiated by nicotine prime gene expression by cocaine. Science Translational Medicine. 2011;**3**(107):107-109. DOI: 10.1126/scitranslmed.3003062

[34] Ruffle JK. Molecular neurobiology of addiction: What's all the (Δ) FosB about? The American Journal of Drug and Alcohol Abuse. 2014;**40**(6):428-437. DOI: 10.3109/00952990.2014.933840

[35] Nestler EJ. Epigenetic mechanisms of drug addiction. Neuropharmacology. 2014;**76**(Pt B):259-268. DOI: 10.1016/j.neuropharm.2013.04.004

[36] Gozen O, Balkan B, Yildirim E, Koçlu EO, Pogun S. The epigenetic effect of nicotine on dopamine D1 receptor expression in rat prefrontal

cortex. Synapse. 2013;**67**(9):545-552. DOI: 10.1002/syn.21659

[37] Simpson DD, Joe GW, Broome KM. A national 5-year follow-up of treatment outcomes for cocaine dependence. Archives of General Psychiatry. 2002;**59**(6):538-544. DOI: 10.1001/archpsyc.59.6.538

[38] Renthall W, Kumar A, Xiao G, Wilkinson M, Covington HE 3rd, Maze I, et al. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron. 2009;**62**(3):335-348. DOI: 10.1016/j.neuron.2009.03.026

[39] Rulten SL, Hodder E, Ripley TL, Stephens DN, Mayne LV. Alcohol induces DNA damage and the Fanconi anemia D2 protein implicating FANCD2 in the DNA damage response pathways in brain. Alcoholism, Clinical and Experimental Research. 2008;**32**(7):1186-1196. DOI: 10.1111/j.1530-0277.2008.00673.x

[40] Hukkanen J, Jacob P 3rd, Benowitz NL. Metabolism and disposition kinetics of nicotine. Pharmacological Reviews. 2005;**57**(1):79-115. DOI: 10.1124/pr.57.1.3

[41] Lee HW, Park SH, Weng MW, Wang HT, Huang WC, Lepor H, et al. E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. Proceedings of the National Academy of Sciences of the United States of America. 2018;**115**(7):E1560-E1569. DOI: 10.1073/pnas.1718185115

[42] de Souza MF, Gonçalves TA, Steinmetz A, Moura DJ, Saffi J, Gomez R, et al. Cocaine induces DNA damage in distinct brain areas of female rats under different hormonal conditions. Clinical and Experimental Pharmacology &

- Physiology. 2014;**41**(4):265-269. DOI: 10.1111/1440-1681.12218
- [43] Johnson Z, Venters J, Guarraci FA, Zewail-Foote M. Methamphetamine induces DNA damage in specific regions of the female rat brain. *Clinical and Experimental Pharmacology & Physiology*. 2015;**42**(6):570-575. DOI: 10.1111/1440-1681.12404
- [44] Tokunaga I, Ishigami A, Kubo S, Gotohda T, Kitamura O. The peroxidative DNA damage and apoptosis in methamphetamine-treated rat brain. *The Journal of Medical Investigation*. 2008;**55**(3-4):241-245. DOI: 10.2152/jmi.55.241
- [45] Berger ND, Stanley FKT, Moore S, Goodarzi AA. ATM-dependent pathways of chromatin remodeling and oxidative DNA damage responses. *Philosophical Transactions of the Royal Society B*. 2017;**372**. pii: 20160283. DOI: 10.1098/rstb.2016.0283
- [46] Li J, Chen J, Ricupero CL, Hart RP, Schwartz MS, Kusnecov A, et al. Nuclear accumulation of HDAC4 in ATM deficiency promotes neurodegeneration in ataxia telangiectasia. *Nature Medicine*. 2012;**18**(5):783-790. DOI: 10.1038/nm.2709
- [47] Massey TH, Jones L. The central role of DNA damage and repair in CAG repeat diseases. *Disease Models & Mechanisms*. 2018;**11**(1). pii: dmm031930. DOI: 10.1242/dmm.031930
- [48] Maiuri T, Mocle AJ, Hung CL, Xia J, van Roon-Mom WM, Truant R. Huntingtin is a scaffolding protein in the ATM oxidative DNA damage response complex. *Human Molecular Genetics*. 2017;**26**(2):395-406. DOI: 10.1093/hmg/ddw395
- [49] Horvath S, Langfelder P, Kwak S, Aaronson J, Rosinski J, Vogt TF, et al. Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. *Aging (Albany NY)*. 2016;**8**(7):1485-1512. DOI: 10.18632/aging.101005
- [50] Glajch KE, Sadri-Vakili G. Epigenetic mechanisms involved in Huntington's disease pathogenesis. *Journal of Huntington's Disease*. 2015;**4**(1):1-15. DOI: 10.3233/JHD-159001
- [51] Lim YW, Sanz LA, Xu X, Hartone SR, Chetin F. Genome-wide DNA hypomethylation and RNA:DNA hybrid accumulation in Aicardi-Goutieres syndrome. *eLife*. 2015;**4**:08007. DOI: 10.7554/eLife.08007
- [52] McKinnon PJ. Genome integrity and disease prevention in the nervous system. *Genes & Development*. 2017;**31**(12):1180-1194. DOI: 10.1101/gad.301325.117
- [53] Reijns MA, Bubeck D, Gibson LC, Graham SC, Baillie GS, Jones EY, et al. The structure of the human RNase H2 complex defines key interaction interfaces relevant to enzyme function and human disease. *The Journal of Biological Chemistry*. 2011;**286**(12):10530-10539. DOI: 10.1074/jbc.M110.177394
- [54] Fousteri M, Vermeulen W, van Zeeland AA, Mullenders LH. Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. *Molecular Cell*. 2006;**23**(4):471-482. DOI: 10.1016/j.molcel.2006.06.029
- [55] Cho I, Tsai PF, Lake RJ, Basheer A, Fan HY. ATP-dependent chromatin remodeling by Cockayne syndrome protein B and NAP1-like histone

- chaperones is required for efficient transcription-coupled DNA repair. *PLoS Genetics*. 2013;**9**(4):e1003407. DOI: 10.1371/journal.pgen.1003407
- [56] Boetefuer EL, Lake RJ, Fan HY. Mechanistic insights into the regulation of transcription and transcription-coupled DNA repair by Cockayne syndrome protein B. *Nucleic Acids Research*. 2018;**46**(15):7471-7479. DOI: 10.1093/nar/gky660
- [57] Wang WY, Pan L, Su SC, Quinn EJ, Sasaki M, Jimenez JC, et al. Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. *Nature Neuroscience*. 2013;**16**(10):1383-1391. DOI: 10.1038/nn.3514
- [58] Alpatov R, Lesch BJ, Nakamoto-Kinoshita M, Blanco A, Chen S, Stützer A, et al. A chromatin-dependent role of the fragile X mental retardation protein FMRP in the DNA damage response. *Cell*. 2014;**157**(4):869-881. DOI: 10.1016/j.cell.2014.03.040
- [59] Khonsari H, Schneider M, Al-Mahdawi S, Chianea YG, Themis M, Parris C, et al. Lentivirus-mediated frataxin gene delivery reverses genome instability in Friedreich ataxia patient and mouse model fibroblasts. *Gene Therapy*. 2016;**23**(12):846-856. DOI: 10.1038/gt.2016.61
- [60] Fujita K, Nakamura Y, Oka T, Ito H, Tamura T, Tagawa K, et al. A functional deficiency of TERA/VCP/p97 contributes to impaired DNA repair in multiple polyglutamine diseases. *Nature Communications*. 2013;**4**:1816. DOI: 10.1038/ncomms2828
- [61] Theil AF, Hoeijmakers JH, Vermeulen W. TTDA: Big impact of a small protein. *Experimental Cell Research*. 2014;**329**(1):61-68. DOI: 10.1016/j.yexcr.2014.07.008
- [62] Koch SC, Simon N, Ebert C, Carell T. Molecular mechanisms of xeroderma pigmentosum (XP) proteins. *Quarterly Reviews of Biophysics*. 2016;**49**:e5. DOI: 10.1017/S0033583515000268
- [63] Serrano M. Epigenetic cerebellar diseases. *Handbook of Clinical Neurology*. 2018;**155**:227-244. DOI: 10.1016/B978-0-444-64189-2.00015-9
- [64] Yandim C, Natisvili T, Festenstein R. Gene regulation and epigenetics in Friedreich's ataxia. *Journal of Neurochemistry*. 2013;**126**(Suppl 1): 21-42. DOI: 10.1111/jnc.12254
- [65] Cvetanovic M, Kular RK, Opal P. LANP mediates neuritic pathology in Spinocerebellar ataxia type 1. *Neurobiology of Disease*. 2012;**48**(3):526-532. DOI: 10.1016/j.nbd.2012.07.024
- [66] Singh A, Compe E, Le May N, Egly JM. TFIIH subunit alterations causing xeroderma pigmentosum and trichothiodystrophy specifically disturb several steps during transcription. *American Journal of Human Genetics*. 2015;**96**(2):194-207. DOI: 10.1016/j.ajhg.2014.12.012
- [67] Le May N, Fradin D, Iltis I, Bougnères P, Egly JM. XPG and XPF endonucleases trigger chromatin looping and DNA demethylation for accurate expression of activated genes. *Molecular Cell*. 2012;**47**(4):622-632. DOI: 10.1016/j.molcel.2012.05.050
- [68] Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *The Journal of Biological Chemistry*. 1998;**273**(10):5858-5868
- [69] Furuta T, Takemura H, Liao ZY, Aune GJ, Redon C, Sedelnikova OA,

et al. Phosphorylation of histone H2AX and activation of Mre11, Rad50, and Nbs1 in response to replication-dependent DNA double-strand breaks induced by mammalian DNA topoisomerase I cleavage complexes. *The Journal of Biological Chemistry*. 2003;**278**(22):20303-20312. DOI: 10.1074/jbc.M300198200

[70] Suberbielle E, Sanchez PE, Kravitz AV, Wang X, Ho K, Eilertson K, et al. Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid- β . *Nature Neuroscience*. 2013;**16**(5):613-621. DOI: 10.1038/nn.3356

[71] Kim JJ, Jung MW. Neural circuits and mechanisms involved in Pavlovian fear conditioning: A critical review. *Neuroscience and Biobehavioral Reviews*. 2006;**30**(2):188-202. DOI: 10.1016/j.neubiorev.2005.06.005

[72] Madabhushi R, Gao F, Pfenning AR, Pan L, Yamakawa S, Seo J, et al. Activity-induced DNA breaks govern the expression of neuronal early-response genes. *Cell*. 2015;**161**(7):1592-1605. DOI: 10.1016/j.cell.2015.05.032

[73] Colón-Cesario M, Wang J, Ramos X, García HG, Dávila JJ, Laguna J, et al. An inhibitor of DNA recombination blocks memory consolidation, but not reconsolidation, in context fear conditioning. *Journal of Neuroscience*. 2006;**26**(20):5524-5533. DOI: 10.1523/JNEUROSCI.3050-05.2006

[74] Hansen N. The longevity of hippocampus-dependent memory is orchestrated by the locus coeruleus-noradrenergic system. *Neural Plasticity*. 2017;**2017**:2727602. DOI: 10.1155/2017/2727602

[75] Halder R, Hennion M, Vidal RO, Shomroni O, Rahman RU, Rajput A, et al. DNA methylation changes in plasticity genes accompany the

formation and maintenance of memory. *Nature Neuroscience*. 2016;**19**(1):102-110. DOI: 10.1038/nn.4194

[76] Duke CG, Kennedy AJ, Gavin CF, Day JJ, Sweatt JD. Experience-dependent epigenomic reorganization in the hippocampus. *Learning & Memory*. 2017;**24**(7):278-288. DOI: 10.1101/lm.045112.117

[77] Bayraktar G, Kreutz MR. The role of activity-dependent DNA demethylation in the adult brain and in neurological disorders. *Frontiers in Molecular Neuroscience*. 2018;**11**:169. DOI: 10.3389/fnmol.2018.00169

[78] Zhang RR, Cui QY, Murai K, Lim YC, Smith ZD, Jin S, et al. Tet1 regulates adult hippocampal neurogenesis and cognition. *Cell Stem Cell*. 2013;**13**(2):237-245. DOI: 10.1016/j.stem.2013.05.006